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# Gene Expression Response to Heat Stress in Two Broiler Lines

## A.S. Leaflet R3100

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### Summary and Implications

The fast growth rate and increased body weight of modern broilers makes them susceptible to heart failure and sensitive to heat stress. To find the genetic causes of these problems, gene expression was measured in hearts of two broiler lines that differed in their response to heat stress. Each line was separated into two groups that were kept under heat stress or at ambient temperature. The comparison of gene expression between the two lines and heat treatments suggests that expression of 325 genes were significantly changed in modern broilers under heat stress, while only 3 genes changed their expression in heritage broilers that adapt well to heat stress. Through identification of genes that regulate cardiac response to heat stress in different broiler lines, we can find potential ways to breed heat-tolerant broilers to mitigate both poverty in Africa and the effects of climate change on the global broiler industry.

### Introduction

Modern broilers with increased growth rate and body weight have dramatically increased meat production in the poultry industry. The broilers' hearts also have additional workload, due to the small size of the heart relative to the body size. Therefore, modern broilers are more sensitive to high temperature and suffer higher mortality from heart failure than other chicken breeds, and it would be beneficial for breeding of heat-tolerant broilers to identify important genes related to cardiac response and tolerance to heat stress in broilers. In this study, we compared the gene expression change in response to heat stress in a modern broiler line (Ross 708) and a heritage broiler line (Illinois) with high throughput RNA sequencing (RNAseq) technology. Illinois is a broiler line under relaxed selection since the 1950s, and has a smaller body weight and higher tolerance to heat stress compared to Ross 708. Therefore, we hypothesize that the two broiler lines would show different responses to heat stress in cardiac gene expression when compared to control group at ambient temperature. The objective of this study is to find potential genes that may regulate cardiac response and tolerance to heat stress through comparison across the 2 lines and 2 treatments.

### Materials and Methods

Twelve male Ross 708 broilers and eleven male Illinois broilers were raised at ambient temperature after hatch. At 21 days-post-hatch (dph), each line was randomly separated to two groups of 5-6 broilers. For the next 21 days, the treatment group was raised under heat stress of 35°C for 7 hours/day, while the control group was kept at ambient temperature. At 42 dph, the left heart ventricles were collected for RNA isolation. All isolated mRNA from the tissue was converted to cDNA for RNAseq. The sequences were analyzed to assess abundance of mRNA for each gene. The genes with significantly higher or lower amount of mRNA in one group than the other were then defined as differentially expressed (DE). Ingenuity Pathway Analysis (IPA) identified enriched gene ontology (GO) and related pathways based on the DE genes.

### Results and Discussion

In the comparison between heat-treatment and control groups, the Ross 708 broilers had 325 DE genes ( $p < 0.1$ ,  $\log_2$  fold-change  $> 1$ ), while only 3 DE genes were found in the Illinois broilers. These findings illustrate the higher sensitivity of Ross 708 broilers to heat stress than the Illinois broilers. For the 325 DE genes in Ross 708 broilers under heat stress, the top GO terms were regulation of cell cycle, cell migration, cell viability and immune cell development. The differential expression of genes related to heart function and disease suggest increased heart rate, heart contraction and hypertrophy of cardiac muscle (Figure 1), which are clinical signs of thermal stress and triggers of heart failure. For the comparison between broiler lines, 45 DE genes were found between the control groups, while 168 genes were found between the treatment groups. These findings indicate that heat stress exaggerated the differences in cardiac function between the two lines. Among the genes with differential response to heat stress between the two lines, the top ranked pathways are mitotic roles of Polo-Like Kinase, cardiomyocyte differentiation via BMP receptors, and factors promoting cardiogenesis in vertebrates. Therefore, the different susceptibility to heart failure under heat stress between the two broiler lines may be mainly due to the regulation of specific pathways. This study provides some potential target genes and pathways for future breeding of heat-tolerant broilers. Under the current circumstances of poor poultry production in hyperthermal Africa and expected global warming, it would be beneficial to develop heat-tolerant broilers.

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### Figure 1. Differentially expressed genes related to cardiac response to heat stress in Ross 708 broiler line.

Green and red color respectively indicates down regulation and up regulation of the differentially expressed genes in the analysis (DUSP5: Dual Specificity Phosphatase 5; ANGPT2: Angiopoietin 2; ATF3: Activating Transcription Factor 3; NTRK2: Neurotrophic Tyrosine Kinase Receptor Type 2; CAVNA2D2: Voltage-Gated Calcium Channel Subunit Alpha-2/Delta-2; VAV3: Vav 3 Guanine Nucleotide Exchange Factor; PNPLA2: Patatin-Like Phospholipase Domain Containing 2; BMP10: Bone Morphogenetic Protein 10; AGTR1: Angiotensin II Type-1 Receptor). The different shapes indicate the different families that the regulators belong to (Triangle: Phosphatase; Square: Growth factor; Oval: Transmembrane receptor; Inverted triangle: Kinase; Rectangle: G-protein coupled receptor; Diamond: Enzyme). The dashed border indicates that the regulator is in extracellular matrix. The orange lines indicate predicted activation that agrees with the literature. The yellow line indicates regulation inconsistent with state of downstream molecules. The grey line indicates the effect not predicted from literature. The color intensity of the function and disease in the lower tier indicates confidence of predicted activation. Octagon and cross shape respectively represents related functions and disease.

